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Changes in spectral reflectance of wheat leaves in response to specific macronutrient deficiency

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Abstract

In wheat (*Triticum aestivum* L.) plants, deficiency of an essential element may drastically affect growth, appearance, and most importantly yield. Wheat, the focus of this study, is one of the crops studied in the CELSS program. Information about nutrient deficiencies in crops grown in controlled environment is essential to optimize food productivity. The main objective of this study was to determine whether deficiency of Nitrogen (N), Phosphorus (P), Potassium (K), Calcium (Ca) and Magnesium (M) alters spectral reflectance properties of wheat leaves. Plants were grown in the greenhouse and growth chamber, in a modified Hoagland's nutrient solution. Spectral reflectance of fully expanded wheat leaves from 280 to 1100 nm, nutrient concentrations (N, P, K, and Ca) and chlorophyll (Chl) were determined when deficiency symptoms were first evident (≈6–7 weeks). Chlorophyll content and fresh and dry weight, were used to assess the severity of the nutrient stress. All nutrient deficiencies affected chlorophyll content and generally increased reflectance in the visible (VIS) 400–700 nm and infrared (IR) 700–1100 nm ranges. Magnesium and nitrogen deficiencies had the most pronounced effect on chlorophyll concentration height, and reflectance. All macronutrient deficiencies tested reduced chlorophyll concentration, increase reflectance in the visible range and caused a shift in the position of the red edge (the point of maximum slope on the reflectance spectrum of vegetation between red and near-infrared wavelengths) toward shorter or longer wavelengths; depending upon the element.

In the greenhouse, N and Mg induced the greatest increase in reflectance of 33% and 25% in the VI range and 86% and 53% in the IR range, respectively. However, in the growth chamber, an increase of 97% and 25% occurred in the VI range, and 20% and 33% in the IR range, respectively. In the IR range in the growth chamber, P, K, and Ca deficiency caused a reduction in reflectance (412–770 nm). This research indicates that mineral deficiencies and reflectance are not specific to one environment and could have important implications for the design of CELSS in space, and perhaps the future of terrestrial agriculture.

Keywords: Wheat; Reflectance; Remote sensing; Spectroradiometer; Macronutrients

1. Introduction

The Human Exploration and Development of Space (HEDS) division of NASA is interested in the production of edible crops on a long term and reliable basis. The Controlled Ecological Life Support System (CELSS) program was developed to understand how life can be maintained in bioregenerative systems during extended spaceflights and extraterrestrial habitations. Wheat, the focus of this study, was one of the crops studied in the CELSS program. Plant production systems developed for use in space will not be able to support large amounts of solid medium because of the costs of launching large quantities of mass (including water)

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into orbit (Wheeler et al., 1990). The nutrient requirements of crops grown in the field may differ from that of crops grown in controlled environments where water, temperature, and nutrient stresses ideally should be minimal. Nutrient composition data could provide feedback to CELSS researchers as they optimize conditions for crop production and modify nutrient composition.

One major factor that would hinder the growth of plants in a system like this is the availability of nutrients. If leaf multispectral reflectance (spectroreflectometry) can be used to detect specific nutrient stresses, it would enable precision supplementation to maintain maximum production without the costs inherent in total replacement of spent nutrient solutions. Changes in the nutrient balance over time can cause even well-designed crop production systems to have problems with deficiencies, or excesses.

Multispectral and hyperspectral imaging systems (i.e., satellites, spectroradiometers, and digital cameras) capture digital images at specific wavelengths of light reflected from plant canopies (Jackson and Pinter, 1986; Baret et al., 1987; Hansen and Schjoerring, 2003; Schuerger et al., 2003; Huang et al., 2004). A variety of spectral measures that relate to nitrogen and chlorophyll content or other plant stresses have been developed (Curran et al., 2001). As leaves become more chlorotic, reflectance increases and the reflectance peak normally centered at about 550 nm, broadens towards the red as absorption of incident light by chlorophyll decreases. These changes are perceived visually as a yellowing of the leaf (Adams et al., 1999). Although the spectral changes in the visible are readily apparent in spectra of stressed vegetation (Baret et al., 1987; Adams et al., 1993), the effects are subtle compared with the changes in the red edge: the sharp increase in reflectance between the red and near infrared (Horler et al., 1983). The robustness and good signal-to-noise ratio of the red edge, in combination with its sensitivity to vegetation, seem to have provided some of the impetus for the development of vegetative indices based on features of the red edge in preference to the visible wavelength range. The red edge is produced by the combination of strong absorption by chlorophyll in the red region and strong reflectance in the IR due to scattering in the leaf mesophyll and the absence of absorption by pigments (Woolley, 1971; Gausman, 1985; Milton et al., 1991). The more commonly used spectral measures of vegetation, which rely on the region of the red edge, have been used to estimate vegetative biomass, productivity, leaf area index, photosynthetic activity or chlorophyll content (Tucker, 1979; Liu et al., 2004). In stressed vegetation the absorption deficiency of the chlorophyll decreases and the IR reflectance decreases due to changes in the cell structure of the plant. This leads to a reduction in reflectance in the IR simultaneous with an increase in reflectance in the red.

Hydroponics has being used in CELSS project to deliver nutrients to the roots of plants. Jones (1997) defines hydroponics as a nutrient solution delivery system, which does not contain any organic or inorganic media for plant support. Hydroponics minimizes problems such as clogged irrigation nozzles, cleaning of culture media between crops and allows for more precise control of the root zone environment. There are endless numbers of nutrient solutions and modified versions thereof, which has been published in the past 50 years (Jones, 1997). The University of California (Berkeley) Agricultural Experimental Research Station bulletin by Hoagland and Arnon (1950) is recognized as the basis for many formulations currently being used by researchers. Depending on the species, type of study, and environmental conditions, adjustments to the solution are made for improved growth or to attain desired results (deficiencies).

System stability and efficiency are paramount in the functioning of a CELSS. Continuous use of a recirculating nutrient solution would be an efficient approach and would promote system stability by avoiding large changes in nutrient uptake in response to plant stresses. Continual reuse of the nutrient solution for growing wheat is an important means of conserving water in a CELSS since the treatment of spent nutrient solution would be costly in terms of space, labor and equipment. Water and space are considered an amenity in any CELSS and could negatively alter the crop's growth and consequently the yield, the proposed recirculating system containing a stable amount of nutrient solution would be a huge advantage. Thus, the proposed work supports accomplishment of one of the critical success factors identified for HEDS, that of developing life support systems for exploration that are significantly less massive and require only a precise resupply of consumables (e.g. Grotenhuis et al., 1997; Jurgonski et al., 1997; Drysdale, 2001; Kitaya et al., 2003; Norikane et al., 2003). The objectives of this research were to: (1) compare the spectral characteristics of normal and nutrient-deficient (–N, –P, –Ca, –Mg, and –K) leaves of wheat grown in hydroponic and vermiculite systems and determine in the reflectance spectrum changes as a function of those deficiencies (2) to study the possibility of early nutrient deficiency detection due to nutrient specific changes in reflectance.

2. Materials and methods

2.1. Growth chamber

Seeds of wheat *Tritlcum aestivum* L. 'Pioneer 2693' were rolled in paper towels, kept moist with distilled water for 24 h in Petri dishes, and then transferred to $10 \times 10 \times 12$ cm green plastic containers containing fine

grade horticultural vermiculite that was fully hydrated with water. Six seeds per container were placed about 3 cm beneath the surface of the vermiculite. Seedlings were thinned to three plants/pot after two weeks. The plants were grown in an environmentally controlled $3.0 \times 2.0 \times 2.5$ m walk-in growth chamber under a mixture of fluorescent and incandescent lighting on a 16/8 h day/night cycle with a photo synthetically active radiance of 250–340 μmol m^{-2} s^{-1}, 25/21 °C day/night temperature and relative humidity of 70/55% day/night during the spring, fall, and winter and a 30/25 °C day/night temperature during the summer.

2.2. Greenhouse

Five wheat seeds were planted into $1.5 \times 1.5 \times 1.5$ cm horticubes (completely pH neutral, foam material without fertilizer built). The plants were grown in the greenhouse under a 16/8 h day/night cycle with a 25/21 °C day/night temperature and relative humidity of 70/55% day/night during fall, spring, and winter, and a 30/25 °C day/night temperature during the summer. When the seedlings had reached 5–6 cm in height, the seedlings were transferred three per/hole into foam plugs in the hydroponic system, consisting of two polyvinyl chloride (PVC) pipes, 10 cm in diameter and 100 cm long, connected at each end to 2 cm PVC pipes. The hydroponic system had five holes, 5 cm in diameter and spaced 17 cm apart on top of a 105 cm long, 10 cm diameter PVC pipe. The PVC pipes and carboys were thoroughly cleaned with a 0.5–1.0% sodium hypochlorite solution (made by mixing one part of household bleach with nine parts of water) to prevent contamination from disease organisms. The nutrient solution was held in 20 L carboys. Carboys were painted silver to prevent exposure of nutrient solution to light to minimize algal growth and pumped or allowed to flow by gravity to the growing pipes. The continuously flowing nutrient solution bathed the roots and then returned to the holding tanks (carboys). To keep the liquid medium circulating and the root system moist, one end of the system was attached a pump controlled by a timer. The pumps ran continuously for 1 h, every 4 h with a flow rate of 1 L^{-1} min. When the pumps were off, the solution flowed back down the fill/drain fittings. The amount of solution that remained in the system was controlled by overflow fittings (located at the input and output entrance of each system) 2 cm in diameter allowing a depth of 6 cm of solution. All systems were connected to air pumps providing continuous airflow through the nutrient medium.

2.3. Induction of deficiency and analysis

Selected nutrient deficiencies were imposed after the initial 20 days of growth. The growth solution consisted of a 100% Hoagland's nutrient solution (Hoagland and Arnon, 1950) with selected components eliminated in certain treatments to induce specific elemental deficiencies (Table 1). The control basal nutrient solution contained the following (mM) NH_4, 10, P, 2200 K, 1000 Ca, 500 Mg, 500 S, 50 Cl, 12.5 B, 0.1 Mo. The micronutrient metals Fe, Zn, Cu, Mn, were added as EDTA (ethylenediaminetetraacetate) chelates. This control solution contained at least (μ)M 20 Fe, 5 Zn, 3 Cu, and 0.3 Mn. The solution was maintained at pH 6.0 ± 0.2 by measuring every three d and adjusting with 1 N H_2SO_4 or 1 N NaOH. When the plants first began exhibiting visual symptoms of nutrient deficiency at about ≈6–7 weeks, data were collected on height, spectral reflectance (250–1100 nm), chlorophyll content, and visual appearance.

Chlorophyll concentration was determined using the Minolta Chlorophyll Meter (SPAD-502). The chlorophyll (in SPAD units) was converted to actual chlorophyll concentrations in mg g^{-1} of fresh leaf tissue using a derived regression equation (Ayala-Silva and Al-Hamdani, 1997). To develop the equation, chlorophyll was extracted using dimethylformamide (DMF)

Table 1
Hoagland nutrient solution with selected components eliminated (− omitted, + included) for induction of deficiency in nitrogen (N), phosphorus (P), potassium (K), calcium (Ca) and magnesium (Mg)

Components of growth solution	Desired mineral (salt) deficiency treatment					
	Complete	–N	–P	–K	–Ca	–Mg
NH_4NO_3	+	–	+	+	+	+
KH_2PO_4	+	+	–	–	+	+
$CaCl_2$	+	+	+	+	–	+
$MgSO_4 \cdot 7H_2O$	+	+	+	+	+	–
KNO_3	+	–	+	–	+	+
Fe(II)-EDTA	+	+	+	+	+	+
H_3BO_4	+	+	+	+	+	+
$ZnSO_4 \cdot 7H_2O$	+	+	+	+	+	+
$CuSO_4 \cdot 5H_2O$	+	+	+	+	+	+
$MnSO_4 \cdot H_2O$	+	+	+	+	+	+
$Na_2MoO_4 \cdot 2H_2O$	+	+	+	+	+	+
$CoSO_4 \cdot 7H_2O$	+	+	+	+	+	+

and analyzed using the method of Ayala-Silva and Al-Hamdani (1997) and then absorbance measured at 647 and 664 nm using a Spectronic 601 spectrophotometer. Spectral reflectance between 280 and 1100 nm was taken from the attached leaves prior to harvest, using a spectroradiometer equipped with an optical cable (field of view of 23°). Reflectance of wheat leaves was obtained on the adaxial area near the midrib. In the greenhouse, data were collected between 1100 and 1300 h to minimize the impact of changing sun angle. Reflectance was calculated as the ratio of reflected to incident radiation, as determined by frequent measurement of reflected radiation from a calibrated white plate. After 90 d of treatment, spectral data were collected, plants were harvested and the shoots were dried at 80 °C for 48 h. The shoot tissue was ground with a Foss Tecator 1093 sample mill to pass a 20-mesh sieve and digested for determination of N, P, Mg, K, and Ca as described by Hu and Barker (1999).

Nitrogen analysis was performed using the LECO FP-528 Nitrogen Analyzer Determinator. Macronutrient (P, Mg, K, and Ca) contents were determined using inductively coupled plasma (ICP, Plasma 400 Analysis Version 4.10). Plants were then classified as either deficient or normal based upon expected normal values for each nutrient (Jones et al., 1997; Mills and Jones, 1996).

2.4. Data analysis

Treatments were arranged in a completely randomized block design with two blocks and 10 subsamples for each of the six nutrient treatments. The two locations (greenhouse and growth chamber) were analyzed separately using conventional statistics because rates of growth and appearance of symptoms differed in the two environments. Data were statistically analyzed using SAS procedures and reported at the 0.05 level of significance. Significant differences ($\alpha = 0.05$) were calculated using Tukey's Studentized Range (HSD, $P < 0.05$) test. Statistical models (ANOVA and means separation) were performed using the General Linear Model (GLM) procedures of PC-SAS Version 8.0 (SAS Institute, Gary, NC). A correlation analysis using SAS was done to determine if the reflectance at each wavelength was positively or negatively correlated with actual tissue nutrient concentration and chlorophyll concentration.

3. Results and discussion

3.1. Visual symptoms

Visual nutrient deficiency symptoms can be a very powerful diagnostic tool for evaluating the nutrient status of plants. Visual symptoms of N, P, and Mg deficiencies begin with the basal leaves and develop acropetally (Kochian, 2000). Nutrient deficiency symptoms were first observed during the experiment after 45–50 days after treatment (DAT). The symptoms ranged from severe chlorosis in N and Mg deficiency treatments to lesser degree of chlorosis in P, K, and Ca. Most symptoms appeared 70–80 DAT but some (N) appeared as early as 45 DAT. The degree of chlorosis and the date at which leaf symptoms of mineral deficiencies were first detected varied among treatments. Chlorosis in wheat plants deficient in P, K, and Ca was not severe. Interveinal chlorosis of younger leaves is the typical symptom of deficiency, and as the severity of the deficiency increases or continues, chlorosis spreads to older leaves (Marschner, 1995).

Nitrogen and magnesium are essential in the formation of chlorophyll. Chlorosis of the older leaves is the first symptom and may move from older to younger leaves resulting in chlorosis of the entire plant (Jones et al., 1997; Mills and Jones, 1996). Deficiency of Ca, K, and P resulted in only slight chlorosis. These elements are essential for plant growth, however, some explanation for these results obtained with P may be that it is readily available in the seed and the period of treatment was not long enough for the deficiency to become more severe. In addition, P deficiency has the tendency to delay maturity, and P deficient plants, in contrast with those lacking N or Mg, are often dark in color resulting in a lesser degree of chlorosis.

The acceptable range of concentrations for macronutrients in wheat shoots is given in Table 2 (Jones, 1997). Wheat shoots from all treatments in the greenhouse and the growth chamber were collected and analyzed for macronutrient analysis. In all treatments with deficiencies, leaf concentrations of the particular element were significantly decreased. Concentration of non-target elements in leaves also varied with the lack of availability of other minerals, resulting in a decrease or increase in the concentration of those elements compared with normal (control) conditions. Therefore, deficiency symptoms and the morphological and physiological modifications observed in treated plants may not be produced by or characteristic of merely one deficient mineral, but could instead be the result of a combination of several minerals being deficient in the nutrient solution. For example, Mg deficiency caused a greater percent reduction in leaf chlorophyll *a* concentration (by 30% relative to control) than in leaf chlorophyll *b* concentration (15%). This underscores the importance of developing a whole plant, multi-nutrient model to relate spectral characteristics to nutrient content of the plants.

3.2. Height, fresh and dry weight

The effect of nutrient deficiencies on shoot height and shoot fresh and dry weight of wheat in the greenhouse and in the growth chamber are shown in Tables 3 and

Table 2
Normal range and actual macronutrient concentration (%) in winter wheat shoots for plants under the influence of different macronutrient deficiency treatments in the greenhouse (GH) and growth chamber (GC)

Location	Range[a]	Minerals				
		Nitrogen, 2.0–3.0	Phosphorus, 0.21–0.50	Potassium, 1.50–3.00	Calcium, 0.21–1.0	Magnesium, 0.16–1.00
Greenhouse	Actual	0.77	0.14	0.49	0.14	0.09
Growth chamber	Actual	0.75	0.24	0.48	0.14	0.15

[a] *Source:* Jones (1997).

Table 3
Mean shoot height (cm) for wheat plants under the influence of different macronutrient deficiency treatments in the greenhouse and growth chamber

Location	Treatments					
	Complete	–Mg	–P	–Ca	–K	–N
Greenhouse	46.75a	43.75ab	43.2ab	42.25abc	42.18abc	28.45d
Growth chamber	45.25a	43.25b	42.5b	41.75bc	42.0 bc	27.75d

Means with the same letter are not significantly different ($P > 0.05$), Tukey's Studentized range (HSD).

Table 4
Mean shoot fresh and dry weight (g) for wheat plants under the influence of different macronutrient deficiency treatments grown in the greenhouse (GH) and growth chamber

Location	Weight	Treatments					
		Complete	–Mg	–K	–P	–Ca	–N
Greenhouse	Fresh	26.53a	24.78b	22.75c	21.66d	21.05d	11.09e
	Dry	12.87a	12.51b	10.45c	10.2c	9.55d	4.58e
Growth chamber	Fresh	24.97a	23.12b	23.05b	22.75c	22.05c	12.5d
	Dry	12.53a	12.51b	12.25b	10.45c	9.55c	5.75d

Means with the same letter are not significantly different ($P > 0.05$), Tukey's Studentized range (HSD).

4. The analysis of variance (ANOVA) means square showed that mineral deficiency treatment significantly affected shoot height; shoot fresh and dry weight for all deficiency treatments in the greenhouse and in the growth chamber. Deficiencies of a particular element showed a reduction on height, fresh and dry weight, however, these reduction varied with the treatments. Plant height was significantly greater in the control treatment relative to plants deficient in N in the greenhouse. Height for plants deficient in Mg, P, Ca, and K treatments ($P > 0.05$) were not different from the control (Table 3). One of the reasons for height not being affected in P deficient plants could have been that P is organically available in the seeds and the period of growth was not long enough to induce deficiency and affect height. In the growth chamber, height of all macronutrient deficient plants was smaller than the control with the most severe reduction in height observed with the N (Table 3). The largest reduction in growth and fresh and dry matter was observed in the N treatment with 40% reduction in height in the greenhouse (Table 3) and 36% in the growth chamber (Table 3). There was a 59% and 62% reduction in fresh weight (Table 4) and 51% and 45% in dry weight (Table 4) from N deficiency in the greenhouse and growth chamber, respectively. These results agree with an earlier report by Zhao et al. (2003).

3.3. Chlorophyll concentration

The ANOVA means square showed that treatment had a highly significant effect on chlorophyll *a* and reflectance; however, the ANOVA means square for chlorophyll *b* indicates that treatment and location had a highly significant effect on chlorophyll *b* and reflectance. In both the greenhouse and the growth chamber, deficiencies of macronutrients decreased the amount of chlorophyll *a* and *b* for all treatments relative to the control ranging from 20% (Chl *a*) and 26% (Chl *b*) for P and K to 35% (Chl *a*) and 41% (Chl *b*) for N (Table 5). The effects of nutrient deficiency on chlorophyll *a* and *b* in the greenhouse and the growth chamber are shown in Table 5. Nitrogen and Mg induced the largest decrease in chlorophyll *a* and *b* (Table 5) followed by P, K, and Ca in the greenhouse. In the growth chamber, similar results were shown with N and Mg deficient plants being the most affected (Table 5) followed by P, K, and Ca. Nitrogen is generally

Table 5
Mean chlorophyll (Chl) concentration for plants under the influence of different macronutrient deficiency treatments in the greenhouse (GH) and growth chamber (GC)

Location	Chl	Treatments					
		Complete	–P	–K	–Ca	–Mg	–N
Greenhouse	Chl *a*	12.96a	10.34b	10.29b	10.05b	8.66c	8.47d
	Chl *b*	6.13a	4.53b	4.23b	3.70c	3.212d	3.169d
Growth chamber	Chl *a*	13.2a	10.35b	10.23b	9.98b	8.52c	7.42d
	Chl *b*	6.05a	4.97b	4.82b	4.76b	4.11c	3.49d

Means with the same letter are not significantly different ($P > 0.05$), Tukey's Studentized range (HSD).

taken up in larger quantities than any other essential element by plants. It combines with C, H, O, and sometimes with S to form chlorophyll, thus a deficiency in N will result in a reduction in chlorophyll. Magnesium is a major essential element and a component of the chlorophyll molecule, therefore a reduction or absence of Mg will result in a reduction in chlorophyll (Marschner, 1995).

3.4. Reflectance

Reflectance spectra between 289 and 1100 nm measured for all treatments are shown in Figs. 1a to 5b. Percent reflectance for treatments in the greenhouse (GH) as well as in the growth chamber (GC) were affected. ANOVA means square for reflectance of N deficient plants indicates that only treatment had a significant effect on N concentration. The ANOVA means square indicates that treatment and location had a highly significant effect on final macronutrient concentrations and reflectance in wheat shoots. Fig. 1(a) shows the percent reflectance of plants deficient in N relative to that of controls in the greenhouse. At the peak reflectance in the visible (VI) range (400–700 nm) values were ≈75% greater for N deficient plants and in the infrared (IR) range (700–1100 nm) ≈97% greater. The slope of the red shift (which is the steep slope between the low reflectance in the visible region (VIS) and the higher reflectance in the near-infrared region (NIR), around 670–780 nm (Imanishi et al., 2004) was steeper for N deficient plants and its midpoint occurred earlier (707 versus 712 nm). The percent reflectance of plants deficient in N in the growth chamber relative to that of controls is shown in Fig. 1(b). At the peak reflectance in the VI range values were ≈97% greater for N deficient plants and in the IR range ≈20% greater. The red edge position (REP), which is the wavelength of the maximum slope in the red edge shifted to a shorter wavelength and the slope was only slightly steeper for N deficient plants and its midpoint shifted back (708 versus 713 nm). This result is in agreement with Mariotti et al. (1996) which confirmed that when plants are under stress, such as nutrient deficiency, there is a loss of chlorophyll, which causes a shift on the red edge position to shorter wavelengths.

The percent reflectance of plants deficient in P in the greenhouse relative to that of controls is shown in Fig. 2(a). At the peak reflectance in the VI range, reflectance for P deficient plants was greater (≈36%) and in the IR range ≈58% greater. The slope of the red shift was steeper for P deficient plants. Its midpoint occurred later (715 versus 712 nm), however, the red shift occurred at a shorter wavelength. Fig. 2(b) shows the percent reflectance of plants deficient in P relative to that of controls in the growth chamber. At the peak reflectance in the VI range for P deficient plants, reflectance was ≈36% greater and in the IR range ≈17% less. The red edge position shifted to a shorter wavelength and the slope was similar for P deficient plants. Its midpoint occurred later (716 versus 713 nm). Similar observations were made by Milton et al. (1991) and Christensen and Jorgensen (2003) while working with soybeans and barley.

Fig. 3(a) shows the percent reflectance of plants deficient in K relative to that of controls in the greenhouse. At the peak reflectance in the VI range, reflectance for K deficient plants was ≈26% greater and in the IR range ≈54% greater. The red shift position occurred at shorter wavelengths. The slope was steeper and its midpoint occurred later (714 versus 712 nm). Fig. 3(b) shows the percent reflectance of plants deficient in K in the growth chamber relative to that of controls. At the peak reflectance in the VI range, reflectance for K deficient plants was ≈59% greater, however, in the IR range ≈2.7% decreased was observed. The slope of the red shift was similar for K deficient plants. Its midpoint occurred earlier (711 versus 712 nm). The percent reflectance of plants deficient in Ca relative to that of controls in the greenhouse is shown in Fig. 4(a). At the peak reflectance in the VI range, reflectance for Ca deficient plants was ≈91% greater and in the IR range ≈31% greater. The location of the red edge shifted to a shorter wavelength, and the slope of the red edge was slightly steeper for Ca deficient plants. Its midpoint occurred earlier (709 versus 712 nm). Fig. 4(b) shows the percent reflectance of

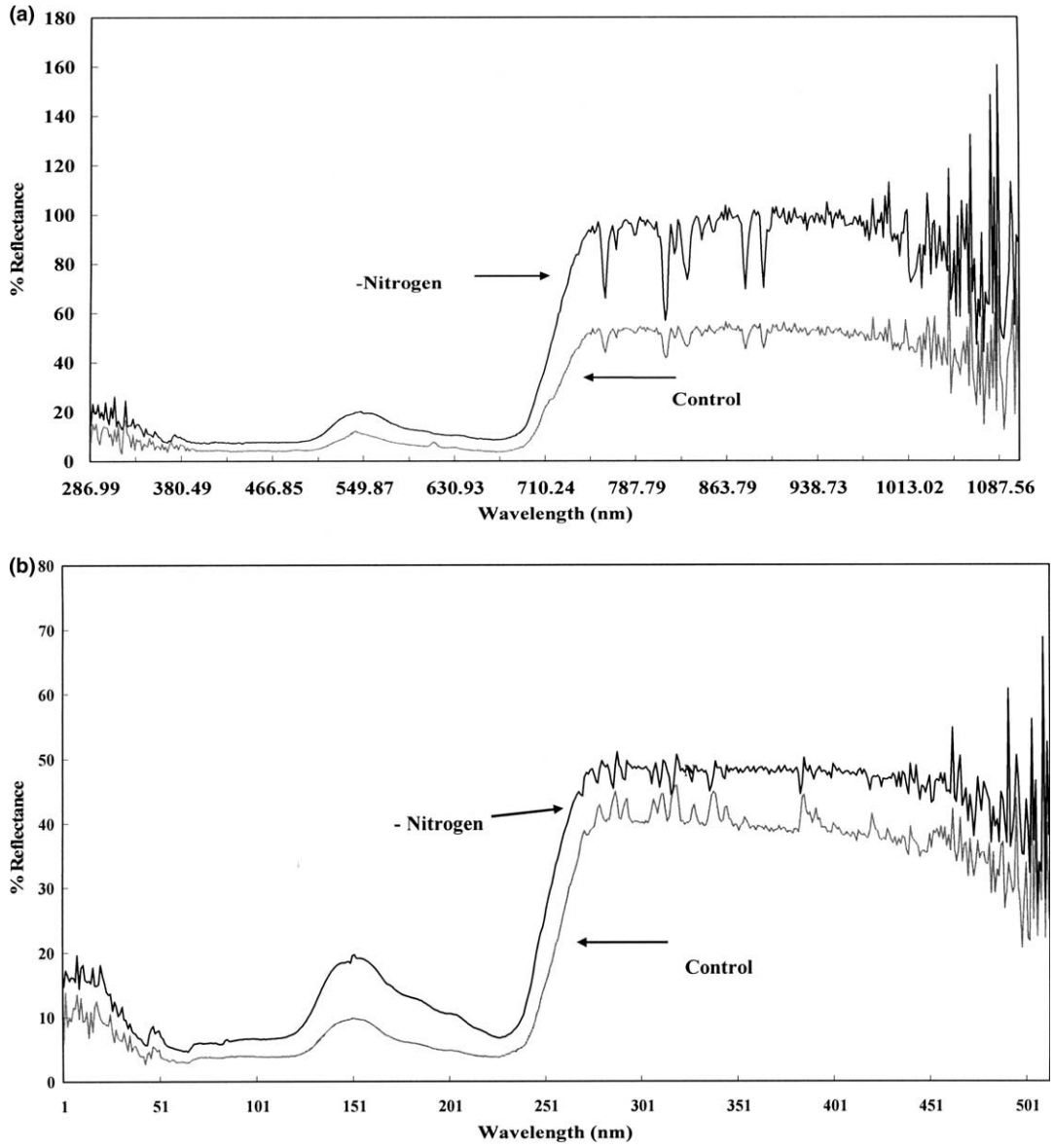

Fig. 1. (a) Percentage reflectance of wheat plants deficient in nitrogen in the greenhouse. (b) Percentage reflectance of wheat plants deficient in nitrogen in the growth chamber.

plants deficient in Ca in the growth chamber relative to that of controls. At the peak reflectance in the VI range, reflectance for Ca deficient plants was 60% greater and in the IR region only ≈3.7% greater. The red edge position shifted to a shorter wavelength and the slope was steeper for Ca deficient plants. Its midpoint occurred earlier (711 versus 713 nm). Fig. 5(a) shows the percent reflectance of plants deficient in Mg relative to that of controls in the greenhouse. At the peak reflectance in the VI range, reflectance for Mg deficient plants was ≈25% greater and in the IR range ≈53% greater. The red edge position shifted to a shorter wavelength and the slope was steeper for Mg deficient plants. Its midpoint occurred earlier (709 versus 712 nm). Fig. 5(b) shows the percent reflectance of plants deficient in Mg relative to that of controls in the growth chamber. At the peak reflectance in the VI range, for Mg deficient plants reflectance was ≈25% greater and in the IR range ≈36%. The red edge position shifted to a shorter wavelength and the slope was slightly steeper for Mg deficient plants. Its midpoint occurred earlier (710 versus 713 nm).

In most cases, an increase in reflectance from 440 to 560 nm and a sharp increase from 675 to 755 nm (red shift) were observed for all deficiency treatments in the greenhouse, however, in the growth chamber a decrease in reflectance in the VI range (P) and in the IR range (K and Ca) was observed. Ponzoni and Goncalves (1999) reported that reflectance values for K and P were significantly lower in the visible range.

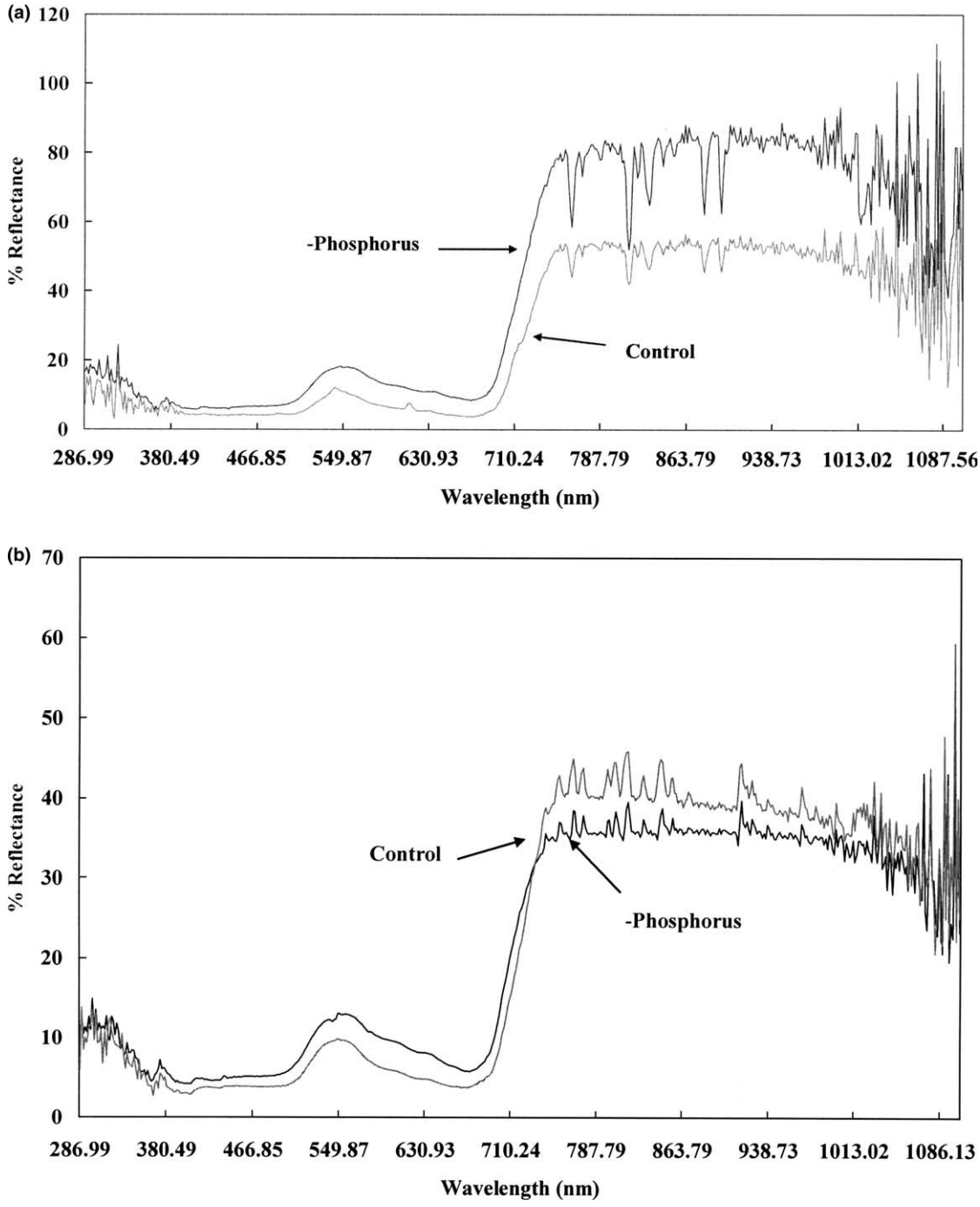

Fig. 2. (a) Percentage reflectance for wheat plants deficient in phosphorus in the greenhouse. (b) Percent reflectance for wheat plants deficient in phosphorus in the growth chamber.

The results of nutrient deficiencies increasing wheat reflectance are consistent with earlier reports by Masoni et al. (1996). All mineral deficiencies caused pronounced modifications in wheat leaf reflectance relative to the control. The least amount of variations were observed in Ca and K deficiencies in the growth chamber. Wavelengths from 413 to 778 nm were correlated with macronutrient deficiencies (data not shown). Huang et al. (2004) shown that there is a correlation between N concentration, chlorophyll content and reflectance. Steep rises in reflectance of vegetation between 650 and 1100 nm are correlated with total chlorophyll concentration and water content (Tucker, 1979; Liu et al., 2004). However, in this experiment,

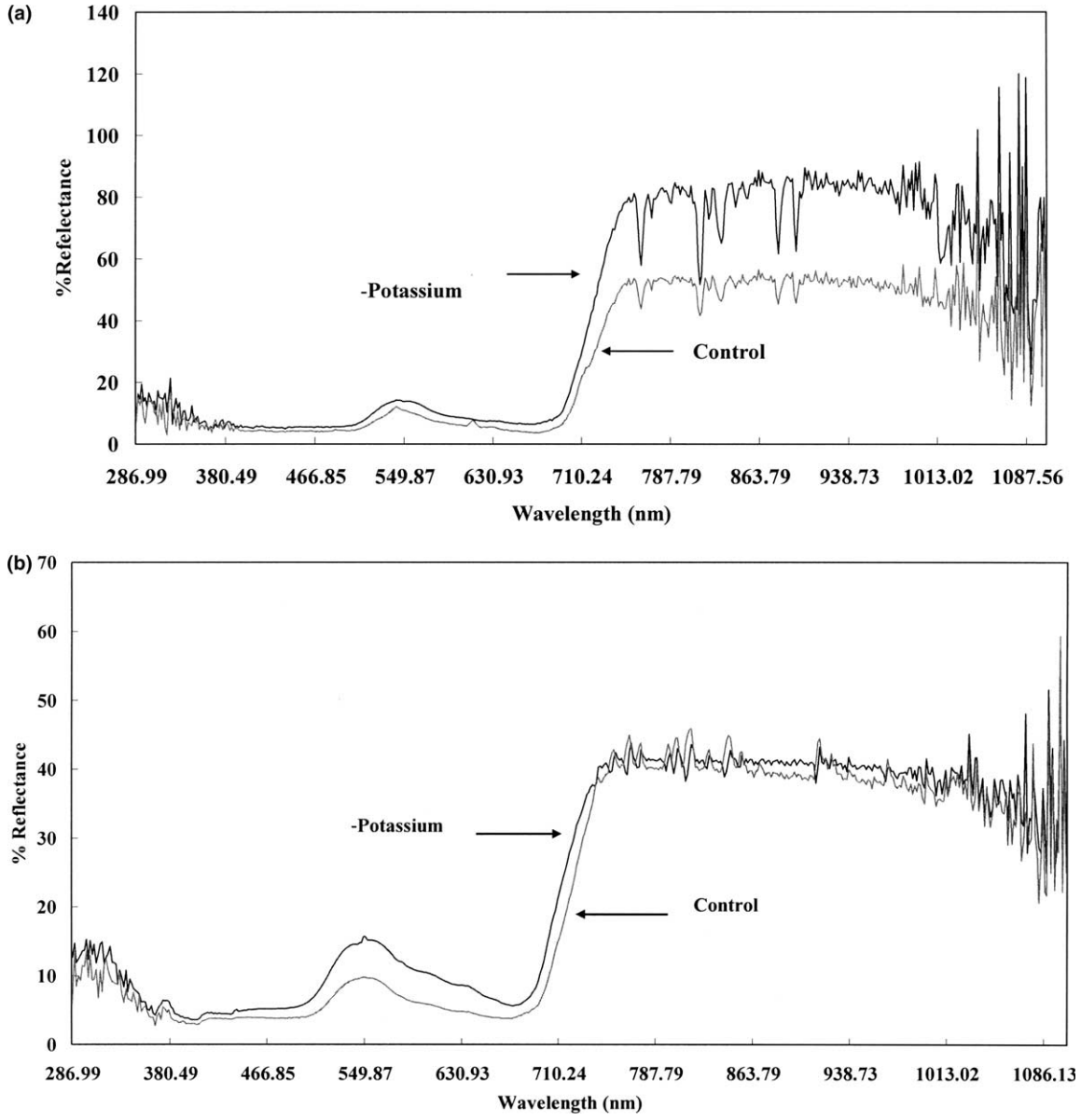

Fig. 3. (a) Percentage reflectance for wheat plants deficient in potassium in the greenhouse. (b) Percentage reflectance for wheat plants deficient in potassium in the growth chamber.

low chlorophyll occurred simultaneously with steep rises. This is well related to the deficiencies in the plants causing chlorosis and senescence of the leave. It is well known that maturity can have an effect on leaf reflectance. Generally older leaves have much higher reflectance than young leaves. Low reflectance in the blue (450 nm) and red region (675 nm) is due to chlorophyll absorption characteristics (Tucker, 1979; Campbell, 1996).

Reflectance dips in the IR at 700–750 nm are associated with strong chlorophyll absorption and leaf cell structure (Boyer et al., 1988). The sharp dip around 764 nm has been associated with absorption by O_2 and water (Gupta et al., 2000; Liu et al., 2004). Gupta et al. (2000) considered near-IR wavelength after 760 nm for detailed study to avoid atmospheric O_2 and H_2O absorption bands and to provide reasonably wider optional bandwidth (s) in the IR region.

3.5. Red-shift position

Typical responses to specific stress (i.e., nutrient deficiency, water stress, O_2 absorption) involve a shift in the red edge to shorter wavelengths occurring between red and near infrared and greater reflectance

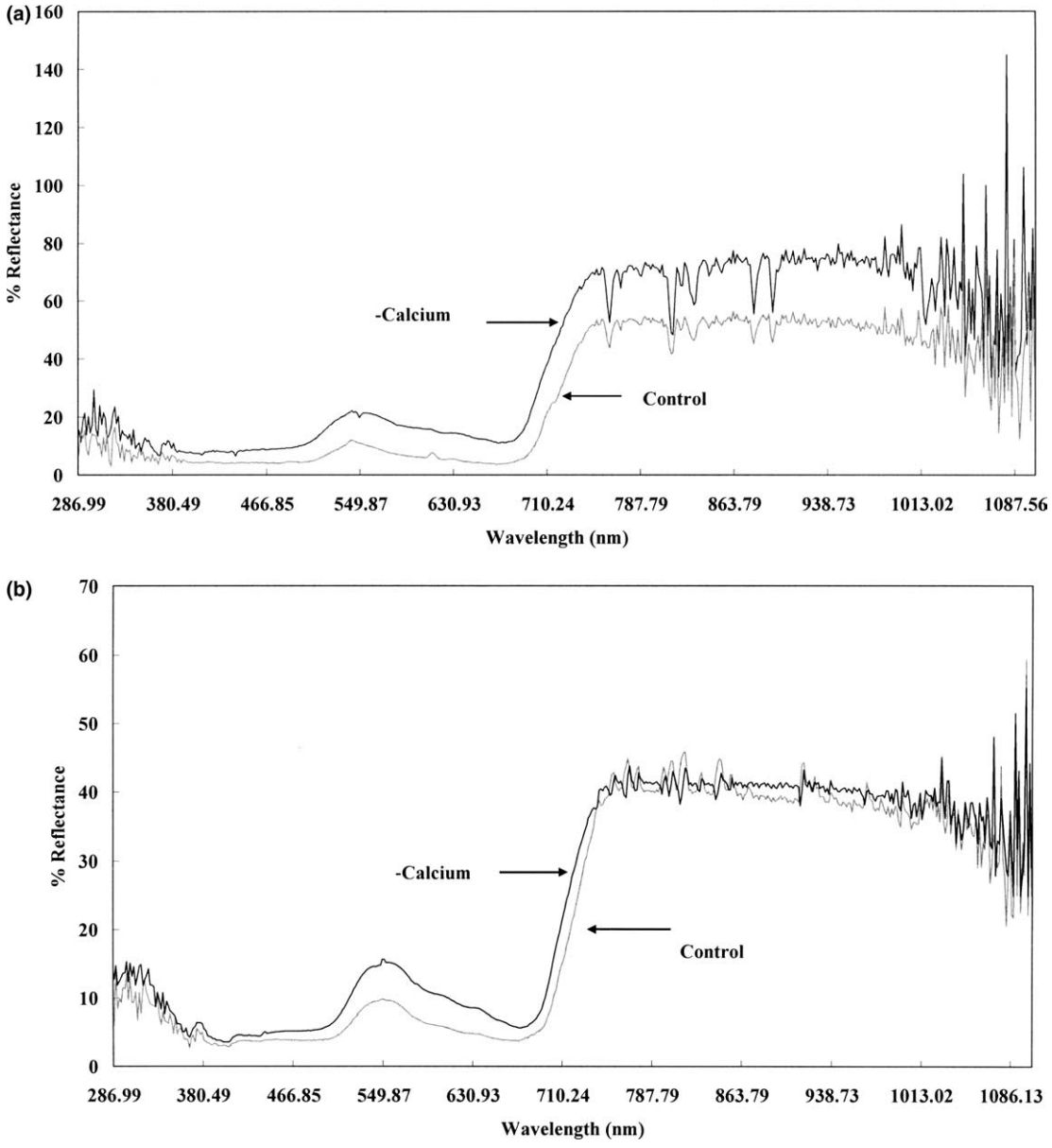

Fig. 4. (a) Percentage reflectance for wheat plants deficient in calcium in the greenhouse. (b) Percentage reflectance for wheat plants deficient in calcium in the growth chamber.

(Curran et al., 1991; Curran et al., 1992; Mariotti et al., 1996; Masoni et al., 1996; Liu et al., 2004; Behrens et al., 2004). All deficiencies caused a change in the red shift position to shorter or longer wavelengths, but this shift in the red edge region was different among treatments. Mariotti et al. (1996) shown that when plants are under stress, such as nutrient deficiency, causes a loss of chlorophyll and a shift of the red edge to shorter wavelengths. Red shifts of 4 nm or greater was observed for wheat grown with N deficient media. With P, K, Ca, and Mg deficiency, the location of the red shift was lesser than 4 nm.

4. Conclusion

Overall, the results of the macronutrient experiment indicate that there is justification for further research in the use of hyperspectral data to determine mineral deficiencies in CELSS crops. Reflectance measurements could be a powerful non-destructive technique to decide on fertilizer application and timely correction of nutrient deficiencies before irreversible damage occurs. In general, nutritional stress caused first a decrease in biomass, leaf chlorophyll concentration and usually an increase in reflectance in the VI and IR range. The

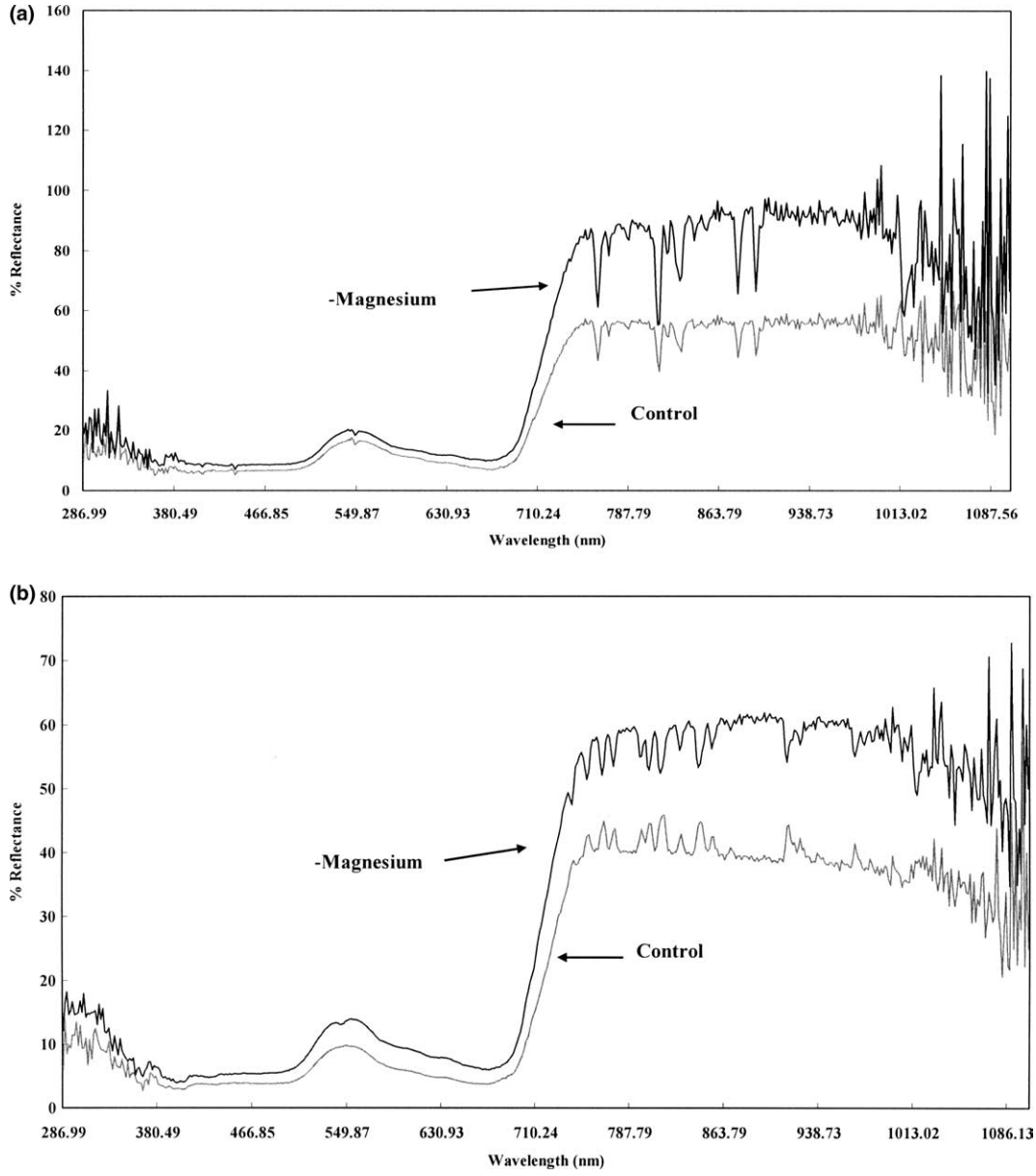

Fig. 5. (a) Percentage reflectance of wheat plants deficient in magnesium in the greenhouse. (b) Percentage reflectance of wheat plants deficient in magnesium in the growth chamber.

red-edge position shifted to shorter wavelengths and had a steeper slope with N and Mg deficiencies. Among the treatments used, variations in spectral properties and red-edge position were proportional to stress levels and leaf chlorophyll concentration, and occurred around the same spectral wavelengths for several nutrients (e.g. N) in particular. Many changes in spectral properties may occur, depending on the interaction between deficiencies of a particular mineral and the level of deficiency. In wheat, for example, the same leaf chlorophyll concentration, or leaf reflectance, or the red-shift position may be found with a deficiency of Fe as with a Mg deficiency. Thus, measurements of spectral properties are useful for detecting early mineral deficiencies in wheat if the specific element deficiency is known but distinguishing among individual nutrients could be difficult.

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